CYTOGENETICS AND BREEDING OF SOME OIL CROPS (SAFFLOWER-CARTHAMUS TINCTORIUS L.)

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C E R T I F I C A T E

This is to certify that the dissertation entitled, "Cytogenetics and Breeding of some Oil crops (Safflower-Carthamus tinctorius L.)", submitted to complement the requirements for the award of the degree of Master of Philosphy in Botany, by Mr. Girish Kumar Shandilya is based on original research carried out under the supervision of late Dr. Zakiul Hasan Zaidi former Reader in the Deptt. of Botany. This work has not been submitted elsewhere for the award of any other degree or diploma.

(Chairman)

A C K N O W L E D G E M E N T

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C O N T E N T

INTRODUCTION

After the start of green revolution and establishment of many Agricultural Universities in India, the country has gained self sufficiency in the field of food grains but it is really shocking that the production of edible oil seeds has not kept pace with the increase production. This is the reason why the vegetable oils are being imported from time to time by India, Such import really is a very sad affair in the history of Indian economy. During the year 1985, 12.81 million oil seeds (oil equivalent = 3.84 million M.T.) was produced in our country and oil seeds worth Rs. 540.98 crores were imported. To check such state of affair, it is the need of time to pay attention towards production of newer varieties of such seeds.

Normal Safflower oil has saponifiable component of about 98-99% comprising glycerides of unsaturated and saturated fatty acids. The percentage of unsaturated fatty acids in Safflower are:

Linoleic acid	approx. 76%
Oleic acid	approx. 16%
Linolenic acid	approx 0.3%

The percentage of saturated fatty acids in Safflower are:

Stearic fatty acid	approx. 6%
Palmitic fatty acid	approx. 6%

It contains unsaponifiable component about 1 which is full of mainly sterols, hydrocarbons such as sequalen, and carotene, fat soluble vitamins A and D, and tocopherols which delay the development of rancidity. One of its (∝- tocopherol) also acts as vitamin E. It contains minute quantities of mucilaginous gums and volatile substance. The vol-atile substance imparts characteristic odour to the oil. There may be some amount of free fatty acids present. The chemical composition of the oils obtained from Carthamus oxyacantha and Carthamus tinctorius are more or less similar. The only difference is: the major liquid acid component in Carthamus oxyacantha is the Oleic acid while in Carthamus tinctorius the lenoleic acid is found. (Anonymous, Wealth of India).

Safflower oil is of great economic importance. This oil is an excellent source of poly unsaturated fatty acids and fat soluble vitamins. It is proved that linoleic acid(the poly unsaturated fatty acid) causes drop in the blood cholestrol level. Thus the safflower oil is the best natural source of this fatty acid. The yellowish colour of the oil is due to carotenoids, because of very low linolenic acid content. The oil has excellent colour retention property. Some of its characteristics are similar to linseed oil and some to soyabean oil (Knowles,1958).

The yield of edible oil of Safflower as compared to other oil crops is comparatively low. The average yield of safflower seed, on world basis is 640 kg/ha only where as average yield of most other oil crops is over one thousand kg/ha (FAO, 1986).

To improve the quality of Safflower oil the higher oleic acid content is desired. In the case of Safflower the Iodine value is the function of the degree of unsaturation linked directly to linoliec acid level. An oil type with lower iodine value is thus desired imporvement.

Keeping in view the economic importance of Safflower a programme of investigation of the following has been proposed to develop hybridization, mutation breeding, induction of polyploidy to develop new high yielding, better quality varieties of Safflower which should be disease and insect pests resistant.

New gene recombination can be brought about by hybridization. The recombination of good quality genes from different varieties and different selected species will produce high desirable cultivars of Carthamus tinctorius . Heterosis has been widely reported in Safflower for yield, oil percentage, plant height and other characteristics brought about.

The use of mutagens(chemical and physical) for induction of mutation is a regular and highly effective

system for crop improvement endeavours (IAEA, 1970).

For qualitative and quantitative traits the polygenic control of Safflower is most useful. This control is most effective for application of mutagens to effect variability.

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2. REVIEW OF LITERATURE

2.1 Origin, Taxonomy and Botany of Carthamus tinctorius

2.1.1 Origin

The cultivated safflower was originated in the area surrounded by the eastern Mediterranean and Persian Gulf (Knowles,1969). The plant is known under cultivation, and is supposed to have originated either from Carthamus lanatus or Carthamus oxyacantha, evidently in two primary centres of origin. Viz Abyssinia and Afghanistan (Anonymous,Wealth of India).

The valid species are distributed from Spain, across North Africa and West Asia to India. Many of them are indigenous to moditerranean region.

This plant has long been domesticated, initially for the orange dye obtained from the florets. It has positively been identified as growing in Egypt 4,000 years ago. Here it was perhaps introduced from Eupharates region. A bundle of single Safflower flowers inserted in packets of willow leaves was found with eighteenth Dynasty mummy of Amenophis I (B.C. 1600).

The carpet weavers of Irano- Afghanistan area used Safflower as a source of dye from ancient times. It was

probably introduced into Southern Russian regions from this area.

2.1.2 Taxonomy and Botany of Carthamus

Genus Carthamus belongs to the tribe cynerae of the family Asteraceae (compositae). It is a shrub grown in Madhya Pradesh, Mysore, Andhra Pradesh, Maharashtra and Uttar Pradesh. The flowers of Carthamus are used as dye for colouring food.

The present taxonomic position of Carthamus tinctorius L. may be stated as follows:

Kingdom	- Plantae
Division	- Herbaceae
Sub division	- Angiospermae
Class	- Dicotyledonae
Family	- Compositae
Tribe	- Cynarae
Genus	- Carthamus
Species	- Carthamus tinctorius

Safflower is a highly branched, herbaceous, thistlelike, annual shrub, varying in height from 30-150 c.m. generally with yellow flowers. The plant has a well defined and frequently fleshy tap-root. It normally produces numerous thin horizontal laterals.

The stem of Safflower is stiff, cylindrical, fairly thick at the base and it becomes thinner as

branching increases. Quite smooth glabrous and light grey or green to white. Brittleness may be genetic characteristics. Varying in degree and age at which it occurs for a brittle steamed type appeared in breeding nurseries at the University of California (Tample & Knowles, 1975). Cultivated Safflower has no true rosette stage as occurs in other Carthamus spp . but the rosette stage can be prolonged when the crop is sown in autumn in the northern hemisphere or inimical weather conditions (Zimmerman, 1973, Ghanavati & Knowles,1977).

The leaves are alternate, rigid and spinescent. Leaf size and shape varies in individual plant. Bendi & Iglisias (1986) observed that the capitulum and leaves of Carthamus lanatus contain vainillic,p-hydroxybenzoic andp-cumaric.

The infloresence is typical of the compositae and consists of numerous florets collected closely together on a circular, somewhat flattend receptacle. The number of florets varies with the variety, the receptacle is surrounded by several layer of involucral bracts.

The flowers are bisexual, actinomorphic, regular, ebracteate, epigynous and complete. Calyx represented by numerous stiff bristles. The corolla colour may be yellow, white or purple. The corolla colour was studied by Narkhade and Deokar (1986). The filament is mostly free and hairy in the middle. The an-ther bases are segittate with the

tatals short and fimbriate. The overy is Bicarpellary & Syncarpous. The style is long.

The fruit achene, resembles a small slightly rectangular Sunflower seed but with the thicker, more fibrous hull.

2.2 Cytology

2.2.1 Chromosome number

Ashri & Knowles (1960), Hanelt (1961), Weiss(1971), Estilai and Knowles(1978) described many species detail. Cultivated Safflower, Carthamus tinctorious has twelve pairs of chromosomes, 2 n = 24 as have Carthamus oxyacantha M.B., Carthamus palaestinus Eig. and Carthamus flavesenes sp. The first three can be readily crossed and yield fertile hybrids. Isoenzyme pattern have confirmed the close genetic relationship between Carthamus tinctorius and Carthamus oxyacantha.

2.2.2 Meiosis

Tapetal cells with chromosame number of 24, 96 and 336 respectively 2x, 8x, 28x were detected in four Safflower varieties. (Chatterjee and Jayaramu, 1981).

2.3 Genetics

Knowles (1982) observed in Safflower from a review of genetic studies and concluded that these characters may be modified: (a) duration of rosette stage,(b) stem length, (c) branching, (d) habit, (e) spininess,(f) stem length, (g) head number, (h) head size, (i) flower morphology (j) mating system (k) seed size (l) hull thickness

(m) oil and protein content and (n) fatty acid composition of the oil.

Gupta & Singh (1988) analysed a data obtained from 10- parent diallel cross and found partial dominance for days to flowering and over dominance in the F_1 to complete dominance in F_2 for days to maturity. Their results from combining ability indicate predominance of additive gene action for two characters.

2.3.1 Male sterility

Male sterility in Safflower has been noticed by Heaton & Knowles (1982). They concluded that male sterility was found in progeny of colchicine treated PI 25394 from Afghanistan. Female fertility was unaffected. The number of seeds in open- pollinated male sterile plant was found less.

Male sterile plants were obtained in the M_2 follwing gamma irradiation (10Kr) of seeds of BS 369. Segregation data in M_3 and M_4 from open pollination submatting or a single dominant gene and that the original M_2 plants were heterozygous (Jambhale and Nerkar, 1985).

2.2.2 Inheritance of yield components

For the number of heads and number of seeds per plant, multiple factor inheritance has been suggested (Kotecha, 1980). Interaction between non-allelic genes was noti in which one combinationwas not found to be significant for both of these traits. There was no association of

these t o characters with seed weights, flower colour and stripped hull. In case of reciprocal crosses between Carthamus tinctorius and Carthamus palaestinus Eig. maternal effects on seed weights were not found existing. It was believed that two loci were involved in inheritance of such traits (Kotecha, and Zimmerman, 1978). Kotecha (1981) has reaffirmed the multiple factor inheritance, lack of maternal effects and epistasis for seed weight.

It has been suggested by Knowles and Yermanos that there are many factors which control the oil content of the seed (Knowles, 1958; Yermanos et. al., 1967). Knowles (1958) suggested that there was negative co-relation between oil content and hull content.

2.3.3. Inheritance of miscellaneous traits

Kotecha (1979) reported that the gene action from the time of flowering (growth period from planting to flowering - GPPF), to maturity (crop period) and the time from flowering to maturity is non-additive. Rao (1982) has re-affiemed non-additive control for days (after sowing) to first flowering and for full maturity.

Immie and Knowles (1970) noted that 'short rosette stage' of growth was controlled by single dominant gene. Pericharak and Kulkarni (1979), however eliminated the consideration of 'short' or 'long' rosette stage and have clarified that rosette formation as such in monogenically controlled, non-rosette growth habit being dominant to 'rosette habit'. Deokar and Patil (1975) upheld the view.

Zimmerman(1976) considered the time of first internode elongation to be responsible for the duration of rosette stage. In addition to major gene for earliness, he also suggested the involvement of a modifier gene.

Internode length and number of nodes are consdered as two components of plant height. It was assumed that perhaps five genes controlled the number of nodes in a cress between Carthamus tinctorius and Carthamus palaestinus (Parichark and Kulkarni, 1972). Abel (1976) taking into account the two characters, ruled out the existence of additive gene action for plant height. However later reports have suggested multiple factor additive mode of inheritance for this character (Kotecha, 1979; Rao 1982). Strong stem is reported to be dominant over weak and twisted stem (Parichark and Kulkarni, 1972).

2.4 Some concept in mutagenesis

2.4.1 Mutagens

It is only eight decade earlier that the idea of inducing mutations and utilizing them for improving cultivated plants come into existance. Hugo-de-varies (1901) for first time gave this idea. It was put to use for the first time by Muller in 1927 when he succeeded inducing certain variations in Drosophilla. Successes with X-rays were achieved by Stadler (1928) in barley and by Goodspeed(1929) in Datura and Nicotiana.

Afterwards several types of ionizing, alpha and beta particles, have been shown to have mutagenic

properties (IAEA, 1970). The only non-ionizing type of radiation carrying mutagenic potential is ultra-violet radiation (IAEA, 1970) of these. Gamma rays are considered more suitable for induction of mutations in plants because they have a short wave length (10^{-13} - 10^{-11} cm).

Heslot (1959), for the first time, demonstrated mutagenic activity of ethylmethane sulphonate (EMS). Rapoport et. at.(1966) discovered nitrosomethyl urea which was found to be very strong mutagen. Later sodium azide (S.A.) was found to be very effective mutagen under certain treatment conditions (Klienhoff et. al. 1974).

2.4.2 Alkylating agents

In practice, however only a few mutagens are used more frequently, most of them belonging to the group of alkylating such as : ethymethane Sulphonate (EMS), di-ethyl-sulphonate (DES), ethylen imine (EI), N-nitroso-N-ethyl urethane (NEU), N-nitroso-N-methyl-urethane(NMU), nitroso-ethyl urea, of these nitroso compounds have been reported to be the most effictive (Rapoport,1962, 1966; Swaminathan, 1966; IAEA, 1970).

A part from easy handling and better efficiency, chemical mutagens have been reported to be more potent in inducing mutations than the physical ones(Sharma, 1965; Blixt and Mossberg, 1967).

2.4.3 Dose effect

Exactly under similar conditions irradiation of differnt

absorbed doses by these different materials has been pointed out (IAEA, 1970). It has been suggested that the frequency of mutations induced by ionizing radiations might be directly proportional to the dose (Stadler, 1931 ; Smith, 1964). Ehrenberg (1955) whowed in peas, positive correlation between dose and effects, like-seedling injury, reduction in rate of survival, with gamma and x-rays and by Blixt (1960) with x-rays and E.I. Germination inhibition was found to be dose depe-ndent (Blixt et. at., 1963) with x-rays. The effect of Gamma irradiation (2-16 K. rad) in Safflower of local C.V., was noted in M_2- M_3 for plant height, number of productive branches and fertile heads and yield per plant (Gayer & Heyab, 1986).

Chemical mutagens like EMS and NMU have been shown to have a dose related in pollen fertility (Narkar, 1970). Siddiqi (1967) found NMU at higher doses to be more effective than gamma rays, EMS and fast neutrons. Micheva (1972) and Salim et. al. (1974) noticed dose dependent reduction in germination, seeling hight and fertility in peas.

2.4.4 Mutagenic Sensitivity

It is well known that the same mutagen dose can cause different degrees of effect in different species. Varied mutagenic sensitivity of different genotypes in groundnut was first reported by Gregory (1955) and in peas by

Lamprecht (1956). Siddiqi (1967) used EMS, NMU, gamma rays and neutrons to determine mutagenic sensitivity in rice. He noticed that varieties belonging to *indica* and *japonica* types differed in radio sensitivity. Akbar *et. al.*(1976) concluded that the difference in radio sensitivity, among the rice varieties may be due to difference in their recovery process involving enzyme activity. Nerker (1970) demonstrated variatal difference in the mutagenic sensitivity of *Lathyrus sativus* . Kukimura and Yatou (1986), noticed that the sensitivity of *Carthamus tinctorius* Callus to Gamma irradiation in vitro was less than that of seedlings but greater than that of the seeds.

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CHAPTER- 3

Material and Methods

3.1 Material

3.1.1 Indian Cultivated variety of Carthamus tinctorius

For the experimental work following variety is to be used in order to improve the yield and other attributes:

Variety used	Characteristic feature
'A-1'	Plant height-medium (30-150 cm.) Seed size-bigger(6-9 mm.) Hull - thicker

This variety is well adapted to agroclimatic conditions of Uttar Pradesh (the region including site of this study) and is popular for cultivation in this region..

3.2 Cultivation practices

3.2.1 Field preparation

The field was first ploughed and levelled. It was watered when the soil was somewhat dry. Weeds were removed and 17 beds of 300 x 250 cm. each were prepared.

3.2.2 Mannure and fertilizer

Cowdung mannure at the rate of one Kg. per square meter was given. 5 gm.ammonium sulphate, 5 gm. Super phosphate and 2 gm. of muriate of potash was mixed in it. Potassic fertilizers and phosphate were sprinkled over

the soil before ploughing. Afterwards ammonium sulphate was applied on the 30th , 55th and 77th days after sowing. Soil and organic mannure was applied to the seedlings in 1 : 1 ratio. The mixture of super phosphate (5 gm), ammonium sulphate (5 gm.) and muriate of potash (2 gm.) was also added to it.

3.2.3 Sowing

Seeds were sown during mid of October. Four replication of 100 seeds each were planted for every treatment. 25 seeds of different teatment were sown in separate beds of 300 cm. x 250 cm. The distance between seedling was kept 60 cm. and the rows 50 cm. Date and time of the sowing was noted and beds labelled properly, indicating the treatments given to the seeds (in each sowing 100 seeds of different treatments were sown).

3.2.4 Irrigation of field

The fields were irrigated every 4th week. Thus irrigation was done twice during mid of November and mid of December.

3.2.5 Weeding and Hoeing

Weeding of the field was done at monthly intervals. It was accompanied by 8-12 cm. deep hoeing.

3.3 Treatment of seeds with mutagens

Both chemical and physical mutagens were used for inducing mutation. Physical mutagens either singly or in combination with the chemical mutagens may also be used.

3.3.1 Mutagens used

1. Ethyl methane sulphonate (EMS)
2. Methyl methane sulphonate (MMS)
3. Sodiun azide (S.A.)
4. Gamma radiation

3.3.2 Pre-treatment

Healthy seeds of uniform size were sorted out for use in the experiment. The seeds were soaked in distilled water at 30° c ± 1 for 10 hours prior to their treatment with mutagens.

3.3.3 Chemical mutagen

For this purpose, 25 soaked seeds were taken in four replicates of each. These were treated with 0.2%, 0.4%, 0.6% and 0.8% of EMS and 0.008%, 0.016%, 0.024%, 0.032% with sodium azide for 12 hours at 27°c ± 1 (Room temperature). 25 pre-soaked seeds were again soaked in phosphate buffer for 12 hrs. to serve as control.

Seeds treated with chemical mutagens were thoroughly washed in tap water immediately after the treatment to remove mutagen. Irradiated seeds were directly used for planting.

3.3.4 Physical mutagen

4 sets of 25 seeds each were kept in peteridishes. These seeds were respectively exposed to Y-rays for 2,4,6 and 10Kr. of irradiation at J.N. Medical College, Radiology Deptt., Aligarh Muslim University, Aligarh. The seeds were exposed to co^{60} (cobalt 60), which is an

important source of Y-rays.

3.4 Cytological studies

Cytological observation will be done only to the study of microsporogenesis. Suitable bud of mutants and bud of each generation will be fixed in carnoy's fluid (absolute alcohol 6 parts, chloroform 3 parts, and acetic acid glacial one part) for about half an hour. Afterwards those buds will be transferred to a mixture of 3:1 of absolute alcohol and propionic acid saturated with ferric acetate, for a period of 24 hrs. at room temperature. The material will then be washed thoroughly with 70% alcohol and stored in it later.

3.4.1 Study of Meiosis

Meiosis will be studied from acctocarmine and propinocarmine squashes of pollen mother cell. Preliminary observation will be made from temporary slides which would be later made permanent using normal butyl alcohal schedule (Bhaduri and Ghosh, 1954).

3.4.2 Study of pollen size and fertility

Pollen size and fertility will be studied for all generations including parents, control, M_1, M_2, M_3 and their segregants, from fresh pollen samples. One or two anthers will be squashed in 1% solution of acetocarmin and then covered with cover glasses. Stained pollen grains with smooth out line will be taken as fertile while unstained, irregular shaped pollen grains will be taken as

sterile. Diameter of the pollen grain will be measured from the fresh pollen of different plants after mounting them in methyl green glycerine jelly. Pollen morphology will also be studied from these slides.

3.5 Fruit Characters

During the present work following fruit characters, bearing relevance to the quality and oil yield will be noted (for consideration).

(a) Average size of the fruit (length and diameter)

(b) Colour of the fruit.

(c) Average weight of the fruit.

(d) Placenta fruit wall ratio (by volume).

(e) Placenta- fruit wall ratio (by weight).

3.6 Yield

Following factors will be taken into account to determined the yield:

(a) A-verage number of fruit per plant.

(b) Average yield (by weight) of fruit per plant.

(c) Estimated yield (by weight) of fruit per hectare.

3.7 Characters to be studied

The following quantitative and qualitative characters will be studied:

1. Plant height (cm.)

2. Number of branches

3. Fruit length (cm)

4. Fruit width or diameter (cm)

5. Fruit size (Fruit length x fruit width)
6. Fruit number
7. Weight of 1,000 seeds.
8. Pollen fertility(%).
9. Number of seeds per plant
10. Seed weight per plant
11. Fruit weight per plant

3.8 Statistical analysis

With a view to find out the extent of variation induced by mutagens observation were recorded on seedling growth (length of the seedling) in different treatment together with control, which have been subjected to statistical analysis. For this purpose best ten seedlings were taken from each treatment to calculate the range, mean, standard deviation, least standard deviation and coefficient of variation.

Range

The difference between the heighest figure and the lowest figure in a data is called its range and symbolically represented as:

$$R = S - H$$

Here, R = Range
S = Lowest figure
H = Highest figure

Mean ($\bar{X}$)

The arithmatic mean, or simple mean or so called average value, is easily computed by taking the sum of a number of values (X_1, X_2 .....and so on) and dividing the

total number of values (N) involved, thus:

$$\bar{X} = \frac{(x_1 + x_2 \text{ ----------} X_n)}{N} \quad \text{or} \quad \bar{X} = \frac{X}{N}$$

where, x_1, x_2 --------x_n = Observations.
N = Number of observation

Standard deviation (S.D.)

Standard deviation will be utilised to determine statistical significance and calculate correlation coefficient. The standard deviation (S.D.) will be calculated by the following formula for each parameter of stud

$$S.D. = \sqrt{\frac{(\bar{x} - x_1)^2 + (\bar{x} - x_2)^2 + \text{------} (\bar{x} - x_n)^2}{N}}$$

Where, $\bar{x}$ = Mean of the observation involved
x_1, x_2, ----x_n = Observations
N = Number of Observations.

Standard error (S.E.) of Mean:

Standard error will be calculated by using the value of standard error and be expressed in $\pm$ as that of standard deviation. The following formula will be used:

$$S.E. = \frac{S.D. \text{ of Samples}}{\sqrt{N}}$$

Where, S.D. = Standard deviation
N = Number of Observations.

Coefficient of Variation:

The coefficient of variation, in term of percentage

is calculated in the following manner:

$$\text{C.V. } (\%) = \frac{\text{Standard deviation of differences}}{\text{Mean value of the differences}} \times 100$$

OBSERVATIONS

Some preliminary experiments were performed to determine the effect of mutagens (chemical & physical) such as EMS, Sodium Azide and Gamma rays, on the percentage of M_1 generation:

4.1 Percentage of seed germination

Seeds treated with different concentrations of EMS, MMS, S.A. and irradiated seeds were sown in the field having well manured soil. The germination percentage observed in different treatments is given in Table 1. The control as well as treated seeds germinated on 18th day after sowing. In the treated material, in general, germination percentage decreased with increasing doses. It is interesting to note that 0.02%(EMS), 0.2% (MMS), 0.008% (S.A.) and 2 Kr (Gamma rays) of four mutagens, the percentage of germination and survival in M_1 was found heigher as compared to their respective concentrations. Among the mutagens, chemical agents showed more effect that irradiation with maximum reduction in Sodium Azide mutagenesis.

4.2 Effect of mutagens on seedling height

Effect of mutagens on the length of the seedling of 'A-1' was studied.

The seeds of 'A-1' were treated with EMS, MMS, S.A. and Gamma rays. The results on seedling height, presented in Table-2 show that all the mutagenic treatments caused reduction in seedling height. The

reduction in height was pronounced in S.A. treated populations than in the material treated with other three mutagens used. However the dose effects were clear in all mutagens.

4.3 Induction of variability by mutagens used

The Table - 2 revealed that the concentrations ranging from .04% - .08% of EMS showed significant reduction in seedling height. However, insignificant increase in length was also observed at 0.02% EMS.

All the concentrations of MMS brought about insignificant reduction in seedling height.

It is calear from the table - 2 that concentrations (4 Kr, and 10 Kr) of Gamma rays showed reduction in seedling height.

Various concentrations of S.A., used in the present invastigation, brought about significant reduction in height of the seedlings. Maximum reduction in seedling height was observed in S.A. in comparison to other three mutagens (EMS, MMS and Gamma rays).

Table 1 : Effect of different mutagenic treatments on seed germination of Safflower (Carthamus tinctinus L.)

Treatments	'A-1'	
	Seed germination (%)	Survival (%)
Control	80	72
EMS		
.02	76	72
.04	72	68
.06	56	52
.08	44	40
Av. EMS	62	
MMS		
0.2	76	68
0.4	72	64
0.6	60	56
0.8	52	48
Av. MMS	65	

contd. .....

Contd. ..... Table 1.

Treatments	'A-1'	
	Seed germination (%)	Survival (%)
S.A.		
0.008	56	52
0.016	52	48
0.024	48	44
0.032	44	40
Av. S.A.	50	
Gamma rays		
2Kr	64	60
4 Kr	60	56
6 Kr	52	44
10 Kr	44	40
Av. Gamma rays	55	

Table 2 : Effect of different mutagenic treatments on seedling height of Safflower (*Carthamus tinctorius* L.)

Var. 'A-1'

Treatments	Range	Mean ± S.D.	L.S.D.	C.V. (%)
Control	5.2-6.5	6.01 ± 0.48	-	7.98
EMS				
.02	5.3-6.8	6.03 ± 0.48		7.96
.04	5.3-6.3	5.92 ± 0.51	1% = .093	8.61
.06	4.8-6.3	5.48 ± 0.75	5% = .064	13.68
.08	4.3-5.8	4.89 ± 0.68**		13.90
MMS				
0.2	4.4-6.5	6.27 ± 0.32		5.10
0.4	5.8-6.6	5.90 ± 0.69	1% = 1.50	11.69
0.6	5.0-6.1	5.73 ± 0.65	5% = 1.03	11.34
0.8	4.4-5.8	5.43 ± 0.46		8.47

Contd. .....

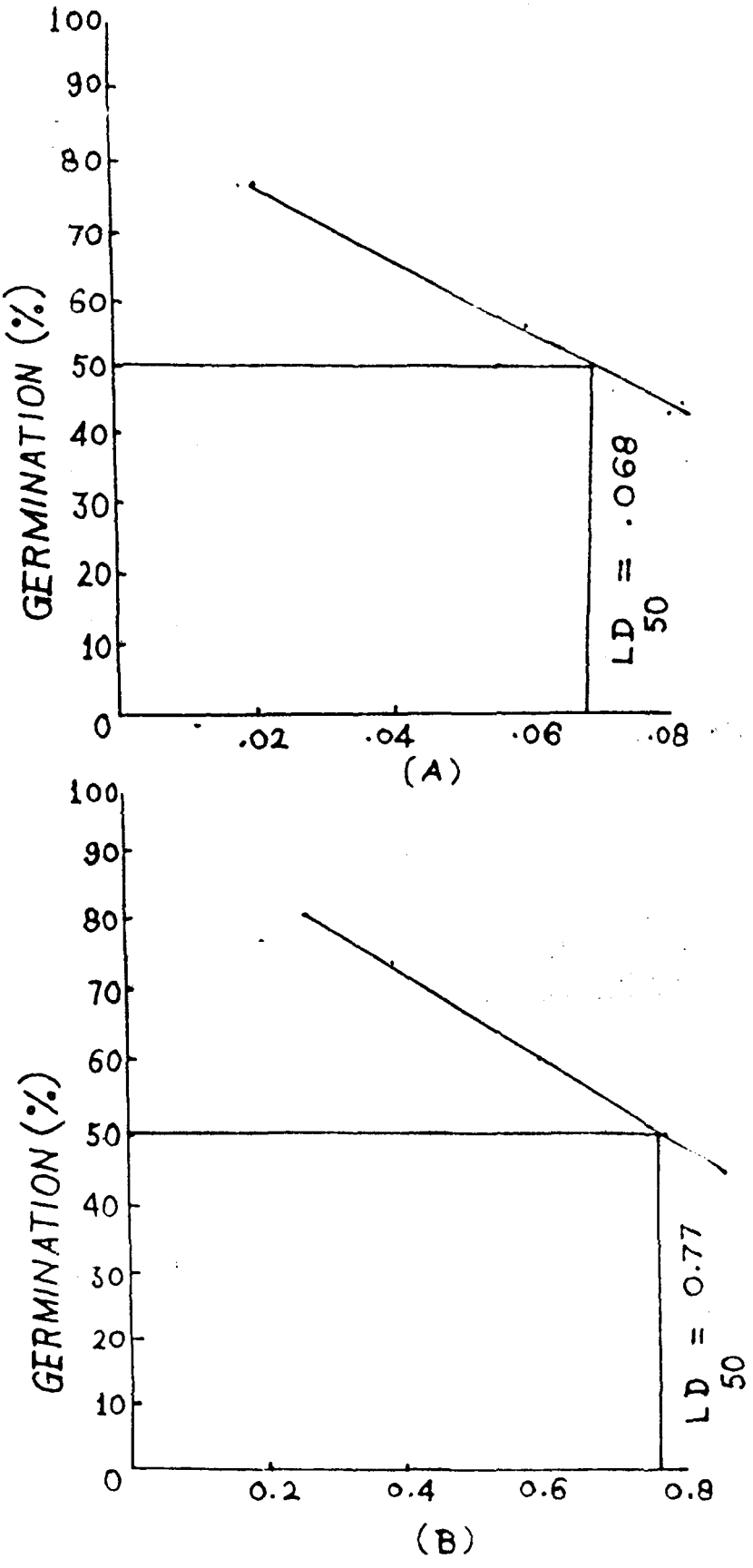
Treatment	Range	'A-1' Mean ± S.D.	L.S.D.	C.V. (%)
S.A.				
.008	4.4-6.2	4.87 ± 0.85**		17.4
.016	3.8-5.8	4.99 ± 0.67**	1% = 0.157	13.58
.024	3.6-5.3	4.16 ± 0.63**	5% = 0.228	15.14
.032	3.3-4.8	3.36 ± 0.62**		16.06
Gamma rays				
2 Kr	5.9-6.2	6.09 ± 0.77		12.64
4 Kr	5.4-6.2	5.32 ± 0.30	1% = 0.516	5.15
6 Kr	4.1-6.1	5.32 ± 1.00**	5% = 0.316	18.83
10 Kr	4.6-5.2	4.92 ± 0.16**		3.25

** Significant at 5% and 1% level.

EXPLANATION OF FIGURES

Fig. A. LD_{50} dose of EMS for the variety 'A-1' of *Carthamus tinctorius* L.

Fig. B. LD_{50} dose of MMS for the variety 'A-1' fo *Carthamus tinctorius* L.



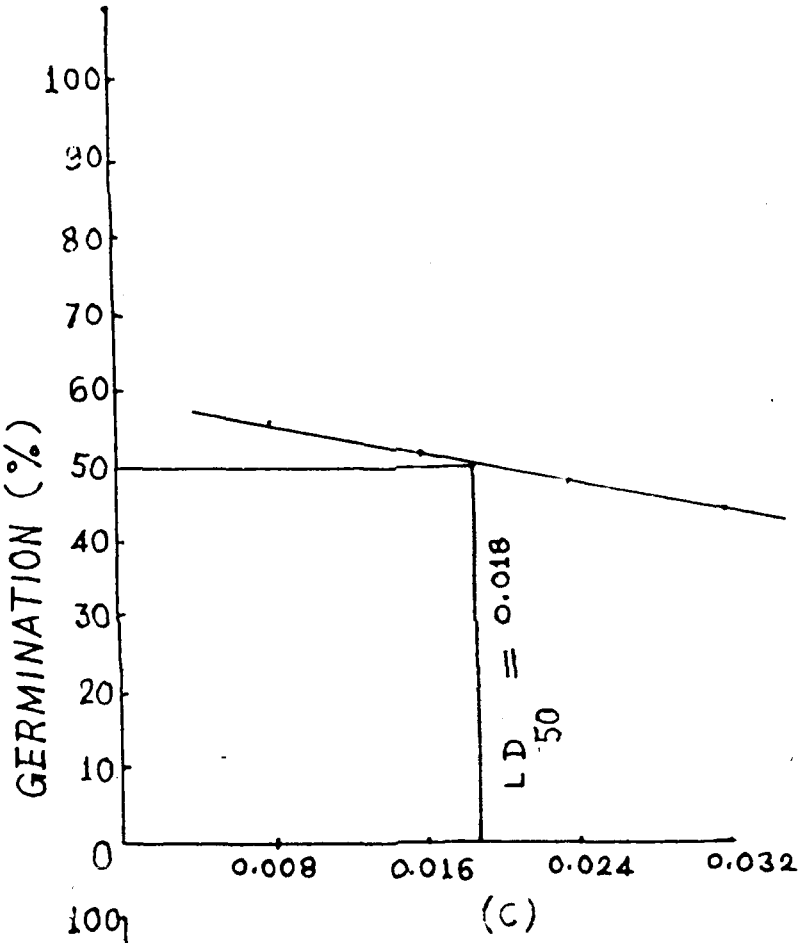
(A) COCENTRATION OF EMS (%)

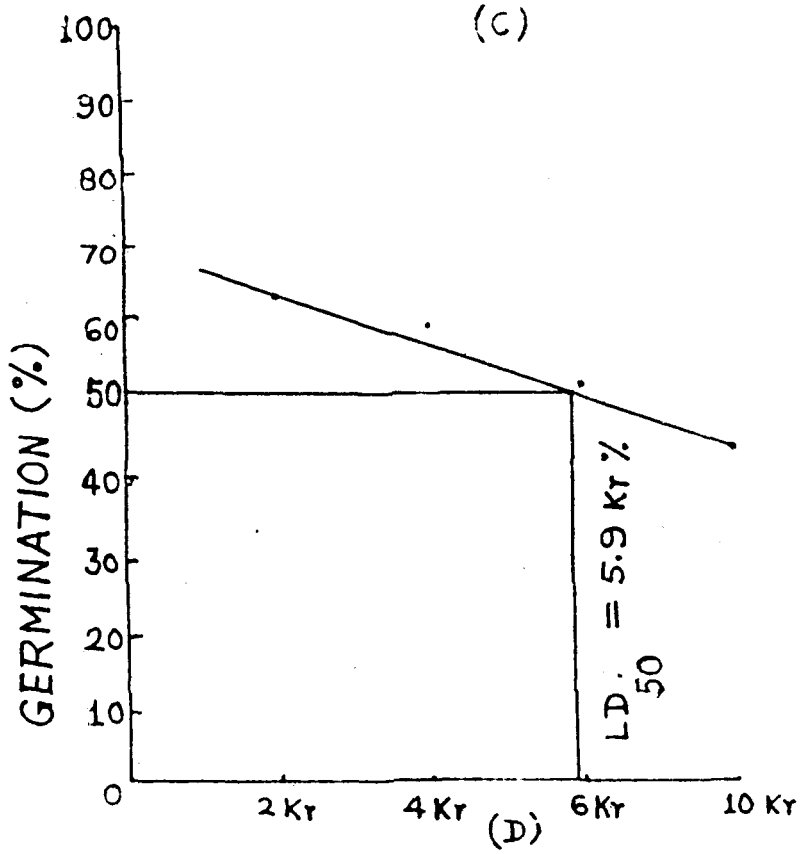
(B) COCENTRATION OF MMS (%)

EXPLANATION OF FIGURES

Fig. C. LD_{50} dose of SA for the variety 'A-1' of *Carthamus tinctorius* L.

Fig. D. LD_{50} dose of Gamma rays for the variety 'A-1' of *Carthamus tinctorius* L.





(C) COCENTRATION OF S.A (%)

(D) COCENTRATION OF GAMMA RAYS (%)

DISCUSSION

In the present study an attempt was made to explore the potential of four mutagens viz. Gamma rays, EMS, MMS and S.A. for creating genetic variability in the variety (A-1). The information obtained may benefit future improvement and endeavours in the course of breeding of Safflower.

The immediate effects of mutagenic treatments as measured by germination and seedling height in the populations emerging from the treated seeds were studied extensively.

For every M_1 parameters sudied, adverse effects of mutagenic treatment were noticed. (Table 1-8). Similar findings have been reported by many workers. (Blixt et al 1963; Singh, 1974; Chauhan and Singh, 1976; Sahu & Kumar, 1978). However Lalikarjunradhya and Channabyregowda (1981) and Ramachandram and Gowd (1983) have reported findings to the contrary, at least in case of germination. They noticed that germination was not significantly affected by mutagenic treatments.

The time taken for germination by seeds is known to be influenced by mutagenic treatment. Van Der Veen and Hildering (1965) recorded that germination delayed when the seeds of tomato were treated with ethyl

methane sulphonate (EMS). No such phenomenon was observed in the present experiment. The treated seeds germinated on the 18th day after sowing like the controls.

Several investigators have reported reduction in germination percentage due to mutagenic treatments; the extent of reduction depending on the type and concentration of mutagens used and the condition of treatment. The percentage of germination has been reported to decrease with increasing concentrations. In the present experiment the percentage of germination of seeds of the variety 'A-1' treated with ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), Sodium azide (S.A.) and Gamma rays was reduced in comparison to controls. A decrease in percentage of seed germination and final survival has also been reported by Chang and Hsieh, 1957; Bhaskaran and Swaminathan, 1961; Goud et. al., 1970; and Ramlu, 1970.

The growth of seedlings were recorded on 30th day after sowing. The height of the seedling decreased with increasing dose of the mutagenic concentrations in 'A-1'. Reduction of seedling growth was observed by Conger and Stevenson (1969) in Hordeum vulgare and Goud et. al. (1970) in Sorghum. A great deal of shoot growth is due to the cell elongation, whereas the root growth is more dependent on cell division (Sinha and Godward, 1972). Reduction of seedling growth brought about by treatment with physical or chemical mutagens has also been reported by several workers (Knozak and Singleton, 1952; Gunckel, 1957 ; Shastry and Ramiah, 1961).

Various explanations have been suggested by different workers for the reduction in seedling growth due to mutagenic treatments. Salim et. al (1974) suggested that accumulation of toxic substances caused physiological disturbances.

The present study has resultod in the isolation and characterization of several mutants which may be useful in the improvement of Safflower. Further studies will be needed to assess their breeding behaviour and practical value.

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CHAPTER - 6

FUTURE PLAN OF WORK

Attempts will be made to obtain improved varities through the induction of mutation. Following experiments will be performed and M_1, M_2, and M_3 generations will be studied thoroughly from the point of view of morphological, cytological, and yield for the purpose of developing improved strains.

6.1 Induction of mutation

Physical and chemical mutagens will be used on dry and presoaked seeds and comparative observations will be madeof morphological characteristics, yield detecting the existance, nature and extent of mutations. Benificial mutations will be isolated and tested for sterility up to M_3 generation.

6.2.1 Induction of mutation using chemical mutagens

EMS, MMS, S.A. were used to induce variability in the M_1 generation. Seeds will be collected from the variants as well as the normal looking plants to proceed forward to M_2 generation.

6.2.2 Induction of mutation using physical mutagens

Dry seeds as well as seeds pre-soaked for 12 hrs. in distilled water will be irradiated with gamma rays

in doses 2,4,6 and 10 Kr respectively. 25 seeds will be taken for each treatment as well as for control.

6.1.3 Combined treatment

Different chemical mutagens will be used in combination with gamma rays for effective induction of mutations. 25 seeds will be used for each treatment. Dry seeds will be exposed to different doses of gamma-rays irradiations of 2,4,6 and 10 Kr. Then these will be soaked in water for 12 hours. After this they will be treated with mutagen solution like those EMS and MMS for 12 hours. These seeds will be thoroughly washed with distilled water and will be sown in sterilized pots.

6.2 Cytogenetics of mutants

Cytogenetic studies of the mutants of M_1, M_2 and M_3 generation will be made specially the number, and behaviour of the bivalents, univalents and tetravalents will be studied.

All studies will be conducted using standard statistical methods of comparision to assertain the population, whether it is homogenous or heterogenous or if particular characterstic is significant or otherwise.

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*Original not seen.